

IMPACTS OF INOCULATION WITH *RHIZOBIUM LEGUMINOSARUM* AND ARBUSCULAR MYCORRHIZAE AND PHOSPHATE ON *VICIA FABA* L. GROWN IN SALT STRESSED SOIL

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Abstract

Effects of AM (arbuscular mycorrhizae), *Rhizobium* and phosphate on the growth of *Vicia faba* L. (faba bean) grown under salinity stress have been studied. *Rhizobium leguminosarum* bv. *viciae* strains RCR 1044 and RCR 1001 mixed culture and AM fungus *Glomus fasciculatum* were inoculated in faba bean seeds. Fifty and 100 mM of NaCl concentrations and rock phosphate and super phosphate as phosphorus sources were used. Percentages of mycorrhizal colonization, nodulation, absolute nitrogenase activity, leghaemoglobin and sodium, potassium, nitrogen and phosphate were assessed. Mycorrhizal colonization of roots decreased by increasing salinity concentration. Mycorrhizal inoculation led to increase in nodule number, fresh and dry weight of nodules, nitrogenase activity and leghaemoglobin contents of nodules. In the presence of mycorrhizae, sodium content decreased, but nitrogen and phosphorus contents increased.

Introduction

Plants, in their natural environment are colonized by external and internal microorganisms. Some of these microorganisms can improve plant performance under stress environments and enhance yield. Arbuscular mycorrhizae (AM) are symbiotic associations with the roots of over 80% terrestrial plant species (Smith and Read 1997) and play an essential role in promoting plant growth. AM promote salinity tolerance by using various mechanisms, such as enhancing nutrient acquisition, producing plant growth hormones, improving rhizospheric and soil conditions and defending roots against soil-borne pathogens. In addition, AM improves host physiological processes like water absorption capability of plants by increasing root hydraulic conduction and favorably adjusting the diffusion balance and composition of carbohydrates (Ruiz-Lozano 2003). Many legumes form tripartite symbiotic associations with nodule-inducing rhizobia and AM, which lead to the acquisition of limiting macro- and micronutrients significantly at the nutrient poor ecosystems and environmental stresses (Marcel *et al.* 2008). Despite the high mycorrhizal growth in the presence of roots, hyphae do not always appear to exhibit 'directional growth' toward the roots till they are very close to the host plant (Mosse and Hepper 1975). Once, contact happens, branching on the root surface takes place, then the fungus forms appressorium on the root surface and enters the root cortex by extending its hyphae, which form arbuscules and vesicles within the cortex.

Salt stress affects plant growth and biomass attributable to non-availability of nutrients and water and the expenditure of energy to counteract the cytotoxic effects of NaCl, also hamper colonization capacity, spore germination and growth of AM hyphae (Jahromi *et al.* 2008). AM associations facilitate plants in salt stress by improving water and nutrient uptake: decrease osmotic potential by increasing accumulation of osmolytes, water-use efficiency, photosynthesis

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and antioxidant production are more efficient in salt-stressed plants associated with AM. The aim of the present work was to study the interaction effects of AM, *Rhizobium* and phosphate on the growth of faba bean grown under salinity stress.

Materials and Methods

Pot experiments were carried out in the Botanical garden of Botany and Microbiology Department, Faculty of Science, Assiut University throughout the season of *Vicia faba* (November-February) under natural conditions of sunshine, temperature, and humidity. Seeds of *Vicia faba* L. (faba bean) cultivar FAAU 103 were surface sterilized by 70% ethanol for 30 s, then soaked for 10 min in 3% H₂O₂ (v/v) and washed 3 times with sterilized distilled water. Five seeds were planted in each plastic pot containing 5 kg autoclaved clay soil. Two concentrations of sodium chloride (50 and 100 mM) were used and replicated three times, the treated soil was inoculated with *R. leguminosarum* bv. *viciae* strain RCR 1044 and RCR 1001 as alone, or with AM (*Glomus fasciculatum*) as a mixed culture. Two representative samples of ground rock phosphate (27% P₂O₅) and processed single super phosphate (15.5% P₂O₅) were added at 0.4 rock phosphate or 0.7 g super phosphate per kg soil. Shoots and roots were harvested 60 days after planting.

Nitrogenase activity was determined on a detached root system, using gas chromatograph with a flame ionization detector equipped with glass columns (5 ft x 1/8 inch) filled with alumina. The excised nodulated roots were placed in 500 ml bottles sealed with a rubber septum. Fifty ml of air were taken and the same volume of acetylene gas introduced into the bottle, incubated at 37°C for 1 hr, then samples from root atmosphere in bottles were withdrawn and injected to the gas chromatograph. Afterwards nodules of each individual root were counted and nodules fresh and dry mass were measured. A calibration curve was constructed using pure ethylene. The percentage of mycorrhizal colonization in root and leghaemoglobin content of nodule cytosol were determined as described as by Abd-Alla *et al.* (2014). Assessment of sodium, potassium, nitrogen and phosphorus contents were determined using Flame photometer technique (Williams and Twine 1960). The data were expressed as mg/g dry matter.

The data were analyzed using one-way analysis of variance (ANOVA) and means of different treatments were compared using the least significant differences LSD at 5% level.

Results and Discussion

Different endomycorrhizal structures have been observed in root samples collected, including arbuscules, hyphae and vesicles that were present between cells of the cortex (Fig. 1). Faba bean FAAU 1003 cultivated in soil treated with rock phosphate showed higher mycorrhizal colonization than that treated with super phosphate (Fig. 2). On the other hand, mycorrhizal colonization decreased by increasing salinity concentrations. The AM association increased the fitness of the host plant by enhancing its survival and biomass and tolerance of biotic or abiotic stresses (Abiala *et al.* 2013). Also, it was reported that AM-inoculated plants grew higher than non-inoculated plants under salt stress (Zuccarini and Okurowska 2008). Giovannetti *et al.* (1996) observed that plant exudates enhanced extensive AM hyphal growth and branching. Increased absorption ability was found to be one of the main factors increasing biomass of mycorrhizal seedlings. On the other hand, a few studies referred to reduction in colonization of plant roots by some AM in the presence of NaCl (Giri *et al.* 2007, Sheng *et al.* 2008) due to the toxic effect of NaCl on the fungi (Giri *et al.* 2007).

Aliasgharzadeh *et al.* (2001) found that the amount of AM spores did not significantly decrease with soil salinity and reported a relatively high spores number, may be due to stimulation of sporulation by high concentration of NaCl (Tressner and Hayes 1971), but spore germination and hyphal growth of AM were inhibited, this causes the accumulation of spores in saline soil (Aliasgharzadeh *et al.* 2001). Jahromi *et al.* (2008) observed that 50 mM NaCl has no significant effect on hyphal length and branched absorbing structures (BAS), though there was a significant decrease in hyphal length and also the number of BAS at 100 mM NaCl.

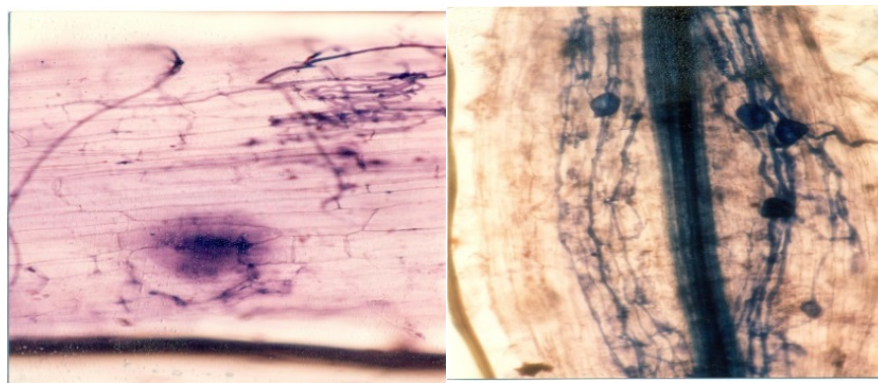


Fig. 1. Light micrographs of arbuscular mycorrhiza *Glomus fasciculatum* hyphae, vesicle and arbuscules colonized roots of *Vicia faba* in the treatment of rock phosphate.

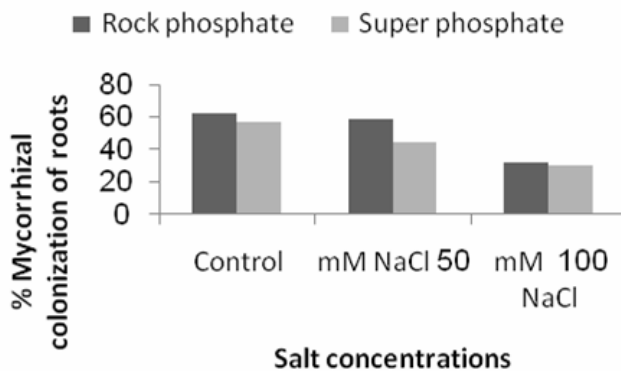


Fig. 2. Percentage of mycorrhizae colonized roots of *Vicia faba* plants inoculated with *R. leguminosarum* by *viciae* strains RCR 1044 and RCR 1001 mixed culture; treated with 50 and 100 mM NaCl salt concentrations of rock and super phosphate.

It was revealed that in the absence of mycorrhizae, the number of nodules decreased as soil salinity increased (Table 1). While, mycorrhizal inoculation of soil treated with salt concentrations increased nodule number compared to control. Fresh and dry weight of nodules reduced by increasing salt concentrations. AM improved mineral content, instead of enhanced biomass production (Johnson-Green *et al.* 2001).

Absolute nitrogenase activity slightly increased by mycorrhizal colonization but in the absence of mycorrhizae, nitrogenase activity decreased with increasing salt concentration compared to control. In the presence of only mycorrhizae, leghaemoglobin content of nodules increased to 37% compared to control. The improvement of nitrogenase activity and leghaemoglobin content by AM might be attributed to facilitating the mobilization of certain nutrients concerned within the synthesis of nitrogenase and leghaemoglobin (Abd-Alla *et al.* 2014).

Table 1. Nodulation, absolute nitrogenase activity and leghaemoglobin content of faba bean plant inoculated with *R. leguminosarum* biovar *viciae* STDF-Egypt and arbuscular mycorrhizal fungus (AM), in the presence of rock phosphate (RP) or superphosphate (SP) and grown on soil treated with two levels of salinity.

Treatments	Nodule			Absolute nitrogenase activity (μ moles C_2H_4/g Fw nodules/h)	Leghaemoglobin content (mg/g Fw nodules)	
	Number	Fresh weight (mg/plant)	Dry weight (mg/plant)			
Control	0 NaCl	45	683.6	90.1	5	3.9
	50 mM NaCl	26	606.1	71	4.17	3.47
	100 mM NaCl	24	340.1	47.9	2.8	2.87
RP	AM	74	1020.67	133.1	6.87	5.2
	AM + 50 mM NaCl	66	932	118.4	6	4.47
	AM + 100 mM NaCl	48	691.7	85.9	4.4	3.43
SP	AM	73	1147	135.7	6.93	5.5
	AM + 50 mM NaCl	68	988.3	121.1	6.33	4.77
	AM + 100 mM NaCl	51	731.9	89.8	4.3	3.37
LSD (at 5% level)	4.382	19.78	4.88	n.s	n.s	

Effect of minerals: It was noted that sodium content of root and shoot of the plant increased with increasing salt concentrations in soil, while inoculation with AM decrease sodium content of the plant (Table 2). Generally, AM increase mineral contents of plants under salt-stress conditions by enhancing uptake of nutrients, this indicates the possibility of a regulative mechanism operative within the plant to contain sodium ions (Ashoori *et al.* 2015), whereas others suggest that AM-colonized plants have lower levels of sodium (Zuccarini and Okurowska 2008), due to the dilution impact owing to growth improvement (Al-Karaki 2006).

Potassium contents of root and shoot were reduced considerably with increasing salinity. Results are in agreement with Ashoori *et al.* (2015). The high salinity causes a major reduction in the quantity of potassium, however mycorrhizal treatments raised potassium content (Giri *et al.* 2007, Zuccarini and Okurowska 2008).

Increased salinity concentrations led to decrease in nitrogen content (Table 2). Application of AM has facilitated assimilation of nitrogen within the host plant, Giri *et al.* 2007 recorded higher accumulation of N in shoots having mycorrhizae than without it.

It is worth mentioning that, phosphate content of bean plant was increased significantly after adding AM to the soil, but the presence of high salt concentrations reduced phosphate content in

the plant (Table 2). Soil salinity considerably reduces the absorption of mineral nutrients, particularly phosphorus, as a result of phosphate ions precipitate with Ca², Mg² and Zn² ions in salt stressed soils and become unavailable to plants (Shokri and Maadi 2009). Increased uptake of P by AM in plants grown under saline conditions could reduce the negative effects of Na and Cl

Table 2. Mineral contents of roots and shoots of faba bean plant inoculated with *R. leguminosarum* var. *viciae* strains RCR 1044 and RCR 1001 mixed culture and AM, in the presence of rock phosphate (RP) or super phosphate (SP) and grown on soil treated with 50 and 100 mM NaCl.

Treatments	Root (mmoleg ⁻¹ dry wt.)				Shoot (mmoleg ⁻¹ dry wt.)				
	Na	K	N	P	Na	K	N	P	
Control	0 NaCl	12.23	8.57	27.43	3.30	11.00	13.13	66.57	4.60
	50 mM NaCl	15.57	7.00	22.60	2.53	14.00	11.23	65.33	3.97
	100 mM NaCl	24.37	5.70	19.00	1.57	24.20	9.20	49.47	3.20
RP	AM	9.23	6.87	44.20	4.40	8.10	18.40	88.90	6.43
	AM + 50 mM NaCl	15.40	5.40	43.60	3.47	14.20	13.40	85.80	5.13
	AM + 100 mM NaCl	21.17	4.97	32.40	2.57	21.12	9.23	66.23	3.73
SP	AM	10.30	7.20	52.97	5.03	8.90	19.80	93.73	6.87
	AM + 50 mM NaCl	16.17	5.10	42.80	4.20	15.07	16.60	94.60	5.43
	AM + 100 mM NaCl	23.37	4.00	30.07	2.97	20.43	9.67	67.17	4.57
LSD (at 5% level)		2.08	0.16	2.81	0.29	2.12	1.19	4.47	0.45

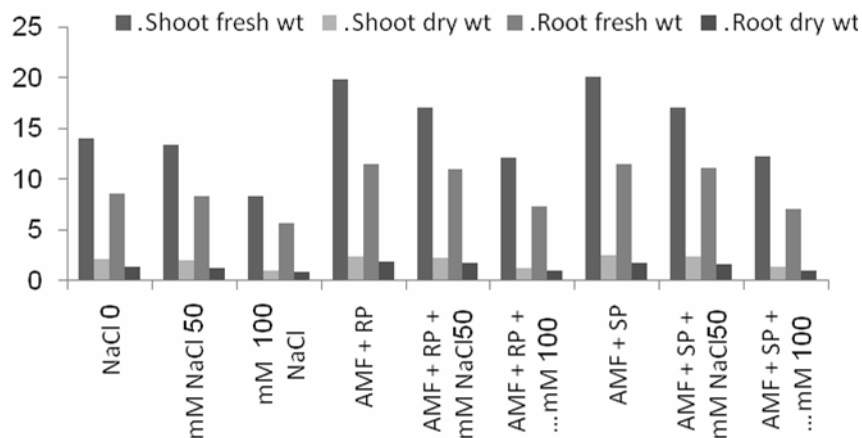


Fig. 3. Fresh and dry weights of each of root and shoot of *Vicia faba* plant treated with 50 and 100 mM NaCl in the presence of AMF inoculated in soil containing rock phosphate (RP) and super phosphate (SP).

ions by maintaining vacuolar membrane integrity, that facilitates compartmentalization inside vacuoles and selective particle intake (Rinaldelli and Mancuso 1996), thereby preventing ions from interfering in metabolic pathways of growth (Cantrell and Lindermann 2001). Mycorrhizae inoculation increased P concentration in plants by enhancing phosphorus uptake facilitated by the extensive hyphae of the fungus that permits them to explore additional soil volume than the non-mycorrhizae plants (Ruiz-Lozano and Azcon 2000). It is estimated that external hyphae deliver up

to 80% of a plant's phosphorus requirements (Matamoros *et al.* 1999). Phosphorus is one of the most difficult nutrients for plants to acquire, in soil, it is found in relatively high amount, however a lot of it's poorly available because of the extreme low solubility of phosphates leading to soil solution concentrations of 10 mM or less and very low mobility (Schachtman *et al.* 1998).

The highest fresh and dry weights of bean plant were observed in the presence of AM, but decreased significantly with increasing salinity (Fig. 3). Mycorrhizal plants maintained higher root and shoot dry weights under given NaCl and P concentrations, it has been reported that mycorrhizal tomato and *Acacia nilotica* plants had higher root and shoot dry weight than the non-mycorrhizal plants (Giri *et al.* 2007).

The present study suggests that the combined application of *Rhizobium* and AM was more effective in plant growth than single inoculation. Additionally presence of AM might be effective against salinity stress and enhance the tolerance of faba bean plants and thus improve the adaptation of plants. Egyptian soil is usually alkaline, thus it is characterized by poor availability of phosphorus, AM enhance uptake of this and alternative elements from soil. The results revealed that the interactive effects of AM, *Rhizobium* and phosphate enhanced growth and mineral uptake of *Vicia faba*.

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